THE EFFECT OF DERIVATIVES OF STILBENE ON THE INSULIN CONTENT OF THE PANCREAS OF THE RABBIT.

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Griffiths and Young (1940) have shown that implantation of tablets of the synthetic oestrogen stilboestrol, 4:4′ dihydroxy-α, β-diethyl stilbene, induces a substantial increase in the amount of insulin in the pancreas of the rat. Shortly afterwards, Vazquez-Lopez (1940), working with mice treated with the natural oestrogen, oestrone, implied that this increase could be correlated with hypertrophy of the Golgi apparatus in all cells of all the Islets of Langerhans examined. Marks and Young (1940) and Griffiths, Marks and Young (1941), using rats again, demonstrated that implanted tablets of the natural oestrogens, oestrone, oestradiol and oestriol, could increase pancreatic insulin. Conrat, Herring, Simpson and Evans (1941) have confirmed the finding that implanted tablets of oestrogen, α oestradiol diproprionate, will increase the amount of insulin in the normal rat's pancreas, but have found that it is ineffective in the hypophysectomized rat whether treated or untreated with maintenance injections of a globulin fraction of anterior pituitary extract.

The above observations, with the exception of those of Vasquez-Lopez, have been made on rats only. Consequently this work was undertaken with the view to investigate the possibility of oestrogen increasing the amount of insulin in another species, the rabbit. The oestrogen used was hexoestrol, 4:4′ dihydroxy-α, β-diethyl-dihydro stilbene. The results are recorded below and also the results of treatment with α methyl stilbene of which Dodds, Fitzgerald and Lawson (1937) have found that even very large doses are non-oestrogenic. The insulin-increasing properties of a third substance, an iodinated oestrogenic derivative of stilbene prepared by Dr. V. M. Trikojus of this department, were examined in one instance. Details of the chemistry of this substance will be published at a later date.

Acknowledgements.

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Methods.

Animals.—Rabbits of both sexes of approximately 2,000 gm. weight were used. Diet consisted of bran, fresh lucerne and water.

Injections.—Each of the three substances was dissolved in arachis oil and injected for periods of from 4 to 10 weeks, in doses of 2·5 to 10·0 mgm. for hexoestrol and the iodinated derivative, and in doses of 10 to 20 mgm. for the α methyl stilbene. In a few cases 5 to 10 mgm. tablets of hexoestrol were implanted (rabbits indicated in Table 1) in addition to the drug being injected in oily solution.

Extraction and Assay of Pancreatic Insulin.—Two blood samples for sugar estimation were taken at the end of treatment, and the animals killed by a blow. A known weight of pancreas composed of pieces taken from various regions of the diffuse organ, was extracted and a crude insulin solution prepared from this extract, by the method of Jephcott (1932). The potency of the unknown solution was determined by a cross-over test on four rabbits, carried out by the method of Marks and Pak (1936). The blood samples for sugar estimation, however, were not pooled, but were taken individually at 1½, 3 and 5 hours after injection of insulin. For conversion of the relative effect to true potency, the writer has used Marks' equation,

$$\log \text{result} = \log \text{assumed strength} + \frac{2}{3}\left(\frac{\text{response to unknown}}{\text{response to standard}} - 1\right)$$

By the above procedure, in two cross-over tests, ½ unit of Burroughs Wellcome's crystalline insulin, tested against a standard of ⅓ unit, returned a potency of 0·46 unit in one case, and 0·48 unit in the other, which indicates a reasonable degree of accuracy.

Blood sugar in all cases was determined by the method of Hagedorn and Jensen on 0·10 ml. of blood (venous).

Results.

From the data in Table 1 it can be concluded that the two oestrogenic substances exerted similar actions on the normal rabbit, namely: no remarkable change in body weight or blood sugar value, a decrease in weight of the liver, marked atrophy of the testes (with retraction from the scrotum into the abdomen) and ovaries, no change in weight of the adrenals, and a very considerable increase in pancreatic insulin. This increase amounted to an increment of 91% above the control value.

TABLE 1.

Rabbit.	Substance Injected and Dose, mgm.	Body Weight, gm.		Blood Sugar, mgm. %.	Liver Weight, gm.	Testis Weight (2), gm.	Adrenal Weight (2), gm.	Insulin Content, Units/gm. of Pancreas.
		Initial.	Final.					
(1) White ♂			2,010	121	56·4	5·79	0·22	3·21
(2) Grey ♀		2,950	2,805	121	52·5	*	0·41	4·98
(3) White ♂		2,270	2,130	87	45·9	3·70	0·43	6·67
(4) White ♂			2,230	103	51·5	2·51	0·25	
(5) White ♂			1,880	78	58·0	5·12	0·27	5·92
Average			2,215	102	53·0	4·28	0·32	5·20
(6) White ♂	Hexoestrol tablets implanted and 20 mgm. injected ..	2,720	2,640	105	41·3	1·66	0·43	6·91
(7) White ♂	Hexoestrol implanted and 27·5 mgm. injected ..	2,890	2,690	116	48·0	1·57	0·39	7·68
(8) Grey ♀	Hexoestrol implanted and 35 mgm. injected ..	2,420	2,270	120	33·9	†	0·23	7·67
(9) White ♂	Hexoestrol 65 mgm. injected	1,750	1,820	82	55·0	0·68	0·24	13·80
(10) White ♂	Hexoestrol 60 mgm. injected	2,150	2,110	65	42·7	1·15	0·24	8·18
Average		2,386	2,306	98	44·2	1·27	0·31	8·85
(11) White ♂	Iodinated derivative 33 mgm. ..	2,220	2,170	112	45·8	1·67	0·24	15·56
(12) White ♂	Methyl stilbene 100 mgm. ..	2,090	2,220	91	56·8	3·16	0·53	7·48
(13) White ♂	Methyl stilbene 400 mgm. ..	2,030	2,150	62	53·2	4·36	0·38	39·69
(14) White ♂	Methyl stilbene 460 mgm. ..	2,450	2,600	99	87·0	5·52	0·49	26·49
Average		2,190	2,323	84	66·0	4·35	0·47	24·49

* Ovary weight (2), 0·60 gm. † Ovary weight (2), 0·22 gm.

The non-oestrogenic α methyl stilbene likewise induced little change in body weight or blood sugar, but produced an increase in liver weight of doubtful significance, increased adrenal weight, no change in testes weights or their disposition in the scrotum, and a markedly enhanced insulin content.

Discussion.

Comparison of the data in Table 1 with the data of Griffiths and Young (1942) and some hitherto unpublished data of the same workers, summarized in Table 2, shows that the responses of the rat and of the rabbit to oestrogen differ in some respects. In the rat there are increases in adrenal and liver weights, whereas in the rabbit, adrenal weights are unaffected and the liver decreases in weight, but in both species body weights and blood sugar values are practically unaltered, the testes atrophy, and pancreatic insulin is increased.

TABLE 2.

Number of Rats in Group.	Treatment.	Blood Sugar, mgm. %.	Liver Wt. gm./100 gm. Body Wt.	Testis Wt. (2), gm./100 gm. Body Wt.	Adrenal Wt. (2) mgm./100 gm. Body Wt.
18	Control	84	3·72	1·07	19·7
20	Two 15-mgm. tablets of stilboestrol implanted for 3–5 weeks ..	93	6·10	0·27	31·9

In Table 2 the blood sugar value for the stilboestrol-treated rats is not significantly above that of the controls, so that neither the writer's results nor those of Griffiths and Young confirm Ingle's (1941) finding that stilboestrol exerts a diabetogenic action.

With regard to the results with α methyl stilbene, the atrophy of the testes in the oestrogen-treated rabbits and the absence of any effect on the testes in those treated with α methyl stilbene, substantiates the finding of Dodds, Fitzgerald and Lawson (1937) that α methyl stilbene is non-oestrogenic. As the α methyl stilbene increases pancreatic insulin and is chemically closely related to hexoestrol and stilboestrol, it would seem that the insulin-increasing properties of the two latter substances have little to do with their oestrogenic properties.

Nothing can be said about the mechanism of action of α methyl stilbene except to point out that Conrat, Herring, Simpson and Evans (1941) have found very good evidence that α oestradiol diproprionate exerts its action through the hypophysis, namely: (1) the pituitaries of oestrogenized rats when implanted raise the pancreatic insulin content of the host animals, while pituitaries of untreated rats do not, and (2) oestrogen fails to increase pancreatic insulin in hypophysectomized rats. It will be of interest to see similar experiments carried out with stilbene derivatives.

Summary.

(1) The synthetic oestrogen 4:4′ dihydroxy-α, β-diethyl-dihydro stilbene and an iodinated derivative of stilbene, increase pancreatic insulin content, and induce atrophy of the testis in the rabbit.

(2) The statement that α methyl stilbene is non-oestrogenic is substantiated in so far as the drug has no effect on the testes of the rabbit. Nevertheless, this stilbene derivative increases to a marked degree the insulin content of the pancreas.

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THE ANTI-INSULIN ACTIVITY OF ANTERIOR PITUITARY AND ADRENAL CORTICAL EXTRACTS.

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(One Text-figure.)

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Selye and Dosne (1939) have shown that in the rat, blood sugar changes brought about by subcutaneously injected adrenalin or insulin are partially inhibited by pretreatment with cortin. Without reference to this work, Jensen and Grattan (1940), Hartman, Brownell, Walther and Edelman (1940), and Grattan and Jensen (1940) have also shown that similar pretreatment antagonizes the hypoglycemic effects of subcutaneously injected insulin in mice. Grattan, Jensen and Ingle (1941) found likewise that cortical extract inhibits the hypoglycemic effects of subcutaneous insulin in adrenalectomized mice and that adrenotropic hormone (Lyons), whilst capable of glycotropic (anti-insulin) activity in normal mice, is without effect in the adrenalectomized animal. They implied that the chronic insensitivity to intravenous insulin, demonstrated by Young (1936) and others, to follow pretreatment with anterior pituitary extract is due to promotion of adrenal cortical secretion by adrenotropic hormone in the extract. Apparently Grattan, Jensen and Ingle were unaware that Himsworth and Scott (1938) demonstrated that Young's pituitary glycotropic factor completely inhibited the hypoglycemic action of intravenously injected insulin in adrenalectomized rabbits.

Furthermore the inhibition of subcutaneously administered insulin by adrenal cortical extract may be due to retardation of the absorption of insulin from the subcutaneous tissues by the adrenal cortical extract. Posterior lobe pituitary extract has been shown to have this effect on insulin injected subcutaneously (Griffiths, 1941). It was considered of interest, therefore, to compare the effects of pretreatment with adrenal cortical extract on the hypoglycemic response to a given dose of insulin injected intravenously, with the effect of the pretreatment on the same dose of insulin given subcutaneously, in the same animal.

Methods.

Unanaesthetized rabbits of both sexes and unanaesthetized male mice were used.

Each rabbit served as its own control, and details of injections in all experiments are given in Table 1 and legends to Figure 1. In the rabbit experiments, two venous blood samples were taken before, and one sample at 10, 20, 30, 60, 90 and 150 minutes after, injection of insulin. In the mouse experiments blood was obtained by decapitation, 40 minutes after injection of insulin. Blood sugar in both cases was estimated by the method of Hagedorn and Jensen on 0·10 ml. blood.

The extracts used were Parke, Davis adrenal cortical extract, Eschatin, containing 0·5% phenol, and a crude extract of fresh ox pituitary anterior lobe made according to Young (1938). These extracts were injected subcutaneously. Burroughs Wellcome's crystalline insulin was used in all experiments.

Results.

The data in Table 1 show that in mice, pretreatment with adrenal cortical or anterior pituitary extract inhibited the hypoglycemic effect of subcutaneously injected insulin. The degree of inhibition in both cases was significant, the difference between the mean values for the groups pretreated with either adrenal cortical or anterior pituitary extract and the mean of the untreated group (injected with insulin) lying at the 2% significance level as determined by Fisher's t-test.

TABLE 1.

Number of Mice in Group.	Pretreatment Injections.	Treatment.	Blood Sugar in mgm. %, 40 Minutes after Final Injection.
8 (Control group)	0·5 c.c. saline subcutaneously at 0, 1½ and 3½ hours during 5-hour fast.	0·5 c.c. saline injected subcutaneously at end of 5th hour.	95
5	0·5 c.c. anterior pituitary extract subcutaneously at 0, 1½ and 3½ hours.	0·1 unit insulin in 0·5 c.c. saline subcutaneously at end of 5th hour.	63
5	0·5 c.c. Eschatin subcutaneously at 0, 1½ and 3½ hours.	0·1 unit insulin in 0·5 c.c. saline subcutaneously at end of 5th hour.	65
6	0·5 c.c. saline subcutaneously at 0, 1½ and 3½ hours.	0·1 unit insulin in 0·5 c.c. saline subcutaneously at end of 5th hour.	50

To a far lesser extent pretreatment with adrenal cortical extract inhibited the action of subcutaneously injected insulin in rabbits. In a series of seven rabbits, eight experiments were carried out, a dose of 2 units of insulin being injected in each case. The average of the percentage reductions (calculated according to Marks and Pak, 1936) was 31·0 without pretreatment, and 26·0 with pretreatment. The difference between these two means was not significant, possibly due to low dosage of adrenal cortical extract. In four experiments, the effect was well marked (Fig. 1, curves A and B), and the difference between these two average curves up to 90 minutes after injection of insulin was significant ($p = 0{\cdot}05$). However, in these same animals there was no glycotropic effect when the same dose of 2 units of insulin was injected intravenously after identical pretreatment (Fig. 1, curves C and D).

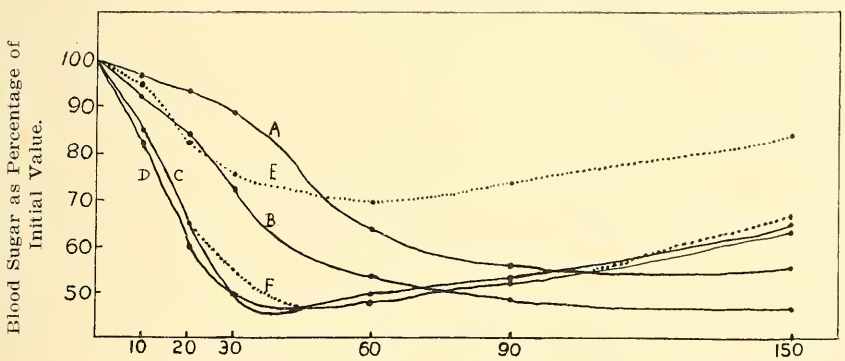

Fig. 1.

Curve A.—Rabbits 3, 5 and 6. Response, at conclusion of 21-hour fast, to 2 units of insulin injected subcutaneously, after pretreatment at 0 and 17 hours of the fast with 50 dog units (2·0 c.c.) Eschatin. Average of 4 experiments.

Curve B.—Same rabbits. Response, at conclusion of 21-hour fast, to 2 units of insulin injected subcutaneously, after pretreatment with 2·0 c.c. of 0·5% phenol at 0 and 17 hours. Average of 4 experiments.

Curve C.—Same rabbits. Response, at conclusion of 21-hour fast, to 2 units of insulin injected intravenously, after pretreatment with 50 dog units Eschatin at 0 and 17 hours. Average of 4 experiments.

Curve D.—Same rabbits. Response, at conclusion of 21-hour fast, to 2 units of insulin injected intravenously, after pretreatment with 2 c.c. of 0·5% phenol at 0 and 17 hours. Average of 6 experiments.

Curve E.—Rabbits 13, 16, 21 and 451. Response, at conclusion of 21-hour fast, to 2 units of insulin injected intravenously, after pretreatment with 4·5 c.c. of anterior lobe pituitary extract at 0 and 17 hours. Average of 4 experiments.

Curve F.—Same rabbits 13, 16, 21 and 451. Response at conclusion of 21-hour fast, to 2 units of insulin injected intravenously. No pretreatment. Average of 4 experiments.

This is in marked contrast to the chronic insensitivity to 2 units of insulin injected intravenously, brought about by pretreatment with anterior lobe pituitary extract (Fig. 1, curves E and F). The difference between those two curves is highly significant ($\rho = 0{\cdot}02$).

Discussion.

It would appear from the above data that the dosage of adrenal cortical extract used in these experiments can exert an antagonism to subcutaneously injected insulin, apparently by lowering the rate of absorption of the insulin, without affecting in the least the response to intravenously injected insulin. This casts doubt on the significance of much of the work on the glycotropic activity of the adrenal cortex, and probably explains why Selye and Dosne found that pretreatment with adrenal cortical extract antagonizes both hyperglycemia due to subcutaneous adrenalin and hypoglycemia due to subcutaneous insulin.

Lewis, Kuhlman, Delbue, Koepf and Thorn (1940) have shown in an unstated number of adrenalectomized dogs that the severe hypoglycemia due to intravenously injected insulin is alleviated by pretreatment with very large doses of adrenal cortical extract. Whether this is due to a true glycotropic action or is due to a general improvement in the animals' health is difficult to say, but should it be accepted as a glycotropic action, the apparent discrepancy between the results of Himsworth and Scott (1938) and Grattan, Jensen and Ingle (1941) may be explained. It is possible that there are two glycotropic factors in pituitary extract; one which exerts its action independently of the adrenals, as was found by Himsworth and Scott, and the other, the adrenotropic factor which Grattan, Jensen and Ingle found would exert a glycotropic action only in animals with intact adrenals, exerting its action by stimulating the adrenal cortex to secrete its anti-insulin substance.

Summary.

(1) Since a dosage of adrenal cortical extract that will exert an anti-insulin effect with subcutaneously injected insulin, has no effect on the same dose of insulin injected intravenously, it is inferred that adrenal cortical extract can retard the rate of absorption of insulin from the subcutaneous tissues. This implies that much of the work on the glycotropic activity of adrenal cortical extract where the insulin was injected subcutaneously, is of doubtful significance.

(2) Other workers have presented data which indicate that possibly the pituitary anterior lobe elaborates two glycotropic substances, one which manifests its action independently of the adrenals, and the other the adrenotropic factor working through the adrenals.

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